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Using Yeast to Discover the Fountain of Youth

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Introduction

The budding yeast *Saccharomyces cerevisiae* has long served as a model organism for the study of basic cellular processes such as transcription, cell cycle regulation, and DNA repair. Its short generation time, well-established molecular genetics, and fully sequenced genome have made this organism a favorite of researchers in diverse fields. Much of the information obtained has been shown to apply to higher eukaryotes, including humans. Recently, researchers have begun using yeast to tackle one of the outstanding questions in science: How and why do organisms age? The identification of individual genes in yeast that can affect the aging process itself has elevated this single-celled fungus to full contender status in the aging field.

The foundation of the study of yeast aging was laid when Mortimer and Johnston discovered that yeast cells have a finite life-span that can be measured ([1](#)). Since then, more than 30 genes have been implicated in yeast aging (see [SAGE KE's Genes/Interventions Database](#)), and at least one molecular mechanism of aging has been established. In this Perspective, we present two fundamentally different measures of aging in yeast: replicative life-span and stationary phase survival (chronological life-span). We describe the benefits and limitations of each and present models that attempt to explain these "aging" phenomena. Finally, we present compelling evidence that the use of yeast as a model system will ultimately prove beneficial to the study of human aging.

Replicative Life-Span

The budding yeast *S. cerevisiae* reproduces by asymmetric cell division, giving rise to an "old" mother cell and a "young" daughter cell. The replicative life-span of a yeast cell is defined as the number of daughter cells produced by a given mother before senescence. In practice, replicative life-span is measured by removing daughter cells from their mothers by micromanipulation after each division and the tallying the number of daughters produced by each mother ([life-span assay](#)). The viability curve for a population of yeast cells measured in this way closely resembles viability curves for other organisms, including humans, and this observation suggests that replicative life-span represents a true aging process ([Fig. 1](#)).

Fig. 1. Viability curves from different model organisms have a similar, characteristic shape. Representative mortality data are shown for *Homo sapiens* (23), *Mus musculus* (23), and *Caenorhabditis elegans* (16). Two different types of aging, replicative (3) and postdiauxic survival (11, and see section on postdiauxic survival), are shown for *S. cerevisiae*.

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The usefulness of yeast as a model system for replicative aging was highlighted by a recent study that demonstrated one of the first molecular causes of aging in any organism. Sinclair and Guarente (2) found that senescence can be caused by the formation and accumulation of extrachromosomal ribosomal DNA (rDNA) circles (ERCs) in mother cells. ERCs are formed by homologous recombination between tandemly arrayed chromosomal rDNA repeats. These circular molecules are self-replicating and asymmetrically segregate to the mother cell, resulting in an exponential increase in ERC copy number with age.

There are several lines of experimental evidence that support a causal role for ERCs in the aging process of yeast. First, mutations that increase the rate of ERC accumulation, such as mutations in the NAD-dependent histone deacetylase *SIR2*, result in a shortened life-span (3). Sir2p promotes silencing at the *HM* loci, telomeres, and rDNA and inhibits rDNA recombination. Second, mutations that decrease the rate of ERC accumulation, such as the deletion of *FOBI*, extend life-span (4). Fob1p is required for the formation of a unidirectional replication fork block in the rDNA. In the absence of Fob1p, rDNA recombination and ERC formation are reduced approximately 10-fold. Taken together, these results demonstrate that ERCs are both necessary and sufficient for wild-type aging. Although it is unclear whether ERCs contribute to aging in other eukaryotes, *SIR2* appears to control longevity in higher organisms (see below).

The ERC model has recently been challenged in two reports. First, Heo *et al.* (5) showed that the short-lived *sgs1* mutant fails to accumulate ERCs more rapidly than wild-type mother cells. This was interpreted to suggest that ERCs do not cause aging. However, the authors failed to distinguish between a mutation that shortens life-span by accelerating the normal aging process and a mutation that shortens life-span by causing a defect unrelated to the normal aging process. In fact, as discussed below, mutation of *SGS1* has recently been shown to result in a high rate of stochastic age-independent mortality (6), which suggests that most *sgs1* mother cells are not dying from old age. A second result that has been purported to weaken the ERC model is the claim that mutation of *SIR2* does not cause an increase in ERC levels (7). In this case, the authors examined ERC levels in an unsorted, logarithmically growing culture of yeast cells. However, when ERC levels were examined in an aged population of mother cells, mutation of *SIR2* was clearly shown to cause a significant increase in ERC accumulation (3).

Postdiauxic Survival: A Model for Postreplicative Senescence?

Yeast aging can also be defined in a chronological sense, based on the ability of cells to maintain viability over time in a nondividing state. When grown on a rich medium such as yeast extract, peptone, and dextrose, yeast cells continue to divide until they have exhausted the nutrient supplies in the medium. At this point, they greatly alter their metabolism and enter a hypometabolic, nondividing state known as stationary phase. The amount of time that cells remain viable in this nondividing state can last on the order of several weeks. When grown on a synthetic medium with glucose as the sole carbon source, yeast

undergo a similar cessation of replication; however, they continue to maintain a high metabolic rate. Cell survival in this postdiauxic state is dramatically reduced relative to stationary phase survival. Interestingly, a postdiauxic survival curve is similar in shape to a plot of replicative life-span ([Fig. 1](#)) and is clearly nonstochastic in nature. This similarity suggests that these two distinct processes may both be pertinent to aging studies.

Postdiauxic survival most closely resembles the aging of postmitotic, differentiated cell populations within a multicellular organism. A large body of research suggests that accumulated oxidative damage caused by reactive oxygen species (ROS) (such as hydrogen peroxide and superoxide anion) is a key determinant of organismal life-span (see [The Two Faces of Oxygen](#)). Treatment with antioxidants has been shown to extend the life-span of *Caenorhabditis elegans* ([8](#)), and neuronal overexpression of enzymes that detoxify oxygen radicals promotes longevity in flies ([9](#)). Likewise, there is evidence to suggest that ROS may limit postdiauxic survival in yeast. One of the primary metabolic changes that occurs upon diauxic shift is a switch from fermentation to respiration. This leads to a concomitant increase in the production of ROS and the associated cellular damage. Detoxification of ROS by superoxide dismutase is required for postdiauxic survival, and overexpression of human Bcl-2 (which stimulates antioxidant protection) increases the survival of wild-type yeast cells ([10](#)).

Recent work suggests that yeast postdiauxic survival may provide a useful model for ROS-induced mortality. By screening for mutants that were resistant to oxidative stress by paraquat, Fabrizio *et al.* ([11](#)) isolated two *S. cerevisiae* strains that showed increased postdiauxic survival. Mutations in two genes, *CYR1* and *SCH9*, were responsible for the increased viability. *CYR1* codes for adenylate cyclase and is an activator of adenosine 3',5'-monophosphate (cAMP)-dependent protein kinase (PKA) activity. Sch9p is a protein kinase with significant homology to *C. elegans* AKT-1 and AKT-2, which function downstream of the longevity regulator DAF-2 in the insulin-like signaling pathway.

In addition to postdiauxic survival, there is also some evidence to suggest that ROS may play a role in the replicative aging process. For example, mutation of *CYR1* also extends replicative life-span by a mechanism resembling caloric restriction ([12](#)). However, in the case of replicative life-span, the effects of *CYR1* are likely to be independent of the stress response pathway activated by *MSN2* and *MSN4*. Msn2p and Msn4p are zinc-finger transcription factors required for transcriptional activation of many stress response genes in yeast. In contrast, the increased postdiauxic survival caused by mutation of *CYR1* requires *MSN2* and *MSN4* ([11](#)), suggesting that *CYR1* may function by two different mechanisms to regulate both types of aging in yeast. A link between replicative aging and oxidative stress also was suggested by Laun *et al.* ([13](#)), who showed that mutation of the superoxide dismutases or catalases of yeast can reduce replicative life-span. Of course, as with any mutation that shortens life-span, it remains to be determined whether this represents a true acceleration of the normal aging process or is simply the result of a detrimental mutation. The critical test of this hypothesis will be to discover an intervention or genetic manipulation that reduces ROS and also extends replicative life-span.

Another interesting parallel between the life-span of nondividing yeast and replicative aging was uncovered by the observation that yeast cells passaged through stationary phase have a reduced replicative life-span ([14](#)). Furthermore, the longer the cells are grown in stationary phase, the shorter their life-span after a return to logarithmic growth. Whether this suggests a common mechanism of aging for postmitotic and mitotic cells is not clear. It will be interesting to discover whether mutations that extend replicative capacity also increase the life-span of postdiauxic cells. In any case, it appears that yeast will be useful as a model to further test the link between ROS and aging and to determine whether similar genetic determinants are important for both replicative life-span and postdiauxic survival.

Yeast Provides Insight About Aging in More Complex Organisms

Critics of the yeast aging field maintain that, as opposed to other model organisms such as *C. elegans* and

mice, yeast is unlikely to yield useful information regarding aging in humans (15). Many argue that the primary cause of aging in multicellular organisms is not the loss of replicative potential, and that the study of replicative senescence in yeast is therefore irrelevant. Those of us who work in the field obviously disagree with this assessment and have been vindicated recently by a series of stunning findings. These results suggest a surprising degree of conservation in the aging process, or at least in the genes that control it, among distantly related organisms.

The most impressive correlation between aging in yeast and higher eukaryotes comes from the work of Tissenbaum and Guarente (16), who found that overexpression of a *SIR2* homolog in *C. elegans* extends life-span in that organism (Fig. 2). Two years earlier, it was shown that overexpression of *SIR2* extends the replicative life-span of yeast cells (3). Strikingly, the mechanism by which *SIR2* extends life-span in each organism appears to be different. The *C. elegans* *SIR2* counterpart, *sir-2.1* acts in the insulin-like signaling pathway known to control aging in worms (16). In yeast, *SIR2* silences gene expression and inhibits recombination in the rDNA repeats. The picture that emerges is that although distinct mechanisms may be at the heart of aging in different organisms, they could still fall under *SIR2* control. It is possible that the nicotinamide adenine dinucleotide (NAD)-dependent histone deacetylase activity of Sir2 proteins (17) allows them to connect metabolic rate to regulation of aging mechanisms in a wide array of contexts. The involvement of *SIR2* in extending yeast life-span by caloric restriction is consistent with this hypothesis.

Fig. 2. A longevity-promoting gene from yeast also functions to extend life-span in higher eukaryotes. Overexpression of *SIR2* extends life-span in *S. cerevisiae* (3). Overexpression of the *SIR2*-homolog *Sir-2.1* extends life-span in *C. elegans* (16).

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Yeast also has served as a useful model for studying the role of RecQ helicases in aging. *S. cerevisiae* has a single RecQ family member, *SGS1*. Mutation of *SGS1* results in a shortened life-span and premature onset of some age-associated phenotypes such as sterility, relocalization of Sir3p to the nucleolus, and nucleolar fragmentation (18). Likewise, mutation of a human RecQ homolog, WRN, results in Werner syndrome, a condition characterized by symptoms of premature aging (see [Of Hyperaging and Methuselah Genes](#)). The Sgs1 helicase has been shown to be important for the survival of yeast cells that are unable to maintain their telomeres (19). Because most human cells also suffer from the problem of shrinking telomeres, the results in yeast suggest that some of the characteristics of cell lines derived from patients with Werner syndrome may be due to defects in telomere maintenance. This view is bolstered by the finding that the expression of telomerase in these cells can rescue them from premature senescence (20). In addition, it was demonstrated recently that the short life-span of yeast *sgs1* mutants may in large part be caused by cell

cycle arrest due to the action of DNA damage checkpoint mechanisms (6). Further dissection of the cellular role of Sgs1p in yeast should allow the formulation of experiments that determine the function of the RecQ helicases in humans.

As mentioned above, postdiauxic survival in yeast may also provide a useful model for the aging of postmitotic cells. The link between ROS and aging in higher organisms is well established. A model for ROS-induced mortality in yeast would give researchers in the field of aging a valuable tool for dissecting the genetic and biochemical pathways involved in this process.

Finally, caloric restriction, which has long been known to extend the life-span of mice, has now been shown to do so in yeast (12, 21) and is allowing the elucidation of the genetics behind this life-span extension. Early studies of aging in yeast implicated the cAMP/PKA pathway as an important determinant of longevity. Mutation of the guanosine triphosphate-binding Ras1 protein increases life-span by up to 40%, and overexpression of Ras2p has a similar effect (22). The mechanism by which the Ras proteins act to regulate longevity is not understood. However, Lin *et al.* (12) recently demonstrated that caloric restriction (through the growth of yeast on low-glucose medium) extends life-span by up to 50% and that this effect can be mimicked by mutations that decrease signaling through the cAMP/PKA pathway. Perhaps these early life-span-extending experiments with the Ras proteins were tapping into this PKA-mediated caloric restriction pathway. As mentioned above, life-span extension by caloric restriction requires Sir2p, which suggests that this protein may play a general role in the relationship between life-span and metabolism.

Conclusions

Researchers who use yeast to study aging must certainly be pleased with the events of the past few years. Not only are we beginning to decipher how various biological components interact to regulate life-span in our favorite fungus, but we are now obtaining the first hints that at least some of these pathways are also important determinants of the rate of aging in distantly related organisms. One task that remains is to determine which of the pathways are specific to yeast aging and which are conserved among more complex organisms. The genetic and biochemical dissection of those that are conserved may someday lead to the development of therapies that allow human beings to lead longer and healthier lives.

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